SEVENTH QUARTERLY SUMMARY REPORT OF PROGRESS

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REDUCTION OF MICROBIAL DISSEMINATION

GERMICIDAL ACTIVITY OF ETHYLENE OXIDE

REDUCTION OF MICROBIAL CONTAMINATION ON SURFACES

EVALUATION OF LEAKAGE OF MICROBIAL CONTAMINATION FROM SPACE SUITS

by the

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Evaluation of Leakage of Microbial Contamination from Space Suits -- Special studies were conducted to develop techniques for measurement of possible leakage into the ambient environment of microorganisms shed by humans wearing a prototype Apollo space suit. Existing static and dynamic shedding chambers used for measuring microbial shedding from humans wearing conventional or hospital garb were adapted for studies with the occupied space suit, pressurized or unpressurized. A technique also was developed for measuring microbial leakage during and following aerosolization of seeded contamination into the unoccupied suit. Various measurements were made over a period of 20 test days.

A technique was devised to estimate shedding arising from expected external contamination of the space suit. Two young, healthy, adult male subjects participated on alternate days for a total of 12 tests. The complete functional suit was donned, including suit undergarments, helmet, and gloves. Immediately after suiting, the external surfaces and hardware of the suit were wiped with sterile gauze sponges saturated with 70% ethyl alcohol. Then, the suited subject was exposed for approximately 4 minutes in a down-flow air-shower. Following this exposure, the subject was placed, unpressurized but sustained by a portable, open-loop, air-cooling system, into an environmentally controlled, dynamic, plastic isolator chamber for 30 minutes. The subjects had last bathed 2 to 6 days prior to these tests. Viable particles shed into the air of the chamber were collected using various types of bacteriological air samplers. Other methods for estimating surface contamination of the suit were not used to prevent any possible damage to the suit.

The mean rates of shedding of airborne microbial contamination obtained on different days are shown in table 1. Variations in viable counts on different days probably reflect differing contamination loads each day or the inability of the subjects to move or exercise in a uniform manner.

Table 1

Microbial Contamination from Occupied, Unpressurized Space Suit During 30-Minute Exposure Periods in a Dynamic Shedding Chamber

Mean Number of Microorganisms Shed Per Minute	
Subject I	Subject II
667	248
87	615
143	122
81	60
49	40
57	
59	
Averages: 163	217

A second technique utilizing the microbiotank (see previous Quarterly Reports for* description and operation of this shedding chamber) was designed to measure the leakage of viable microorganisms from the occupied, pressurized suit into a static environment. The microbiotank was modified with connections for breathing and for telephone communication. Pre-test R. H. in the chamber was reduced to approximately 25% at 68F, but was not monitored during the test period. A suited subject was placed into the tank, connections for breathing and telephone communication were made, and the internal suit pressure was adjusted to 18.4 psia. Sterile air was supplied for breathing and pressurization through an open-loop system at a flow rate of approximately 9 cfm. Test periods were varied from 30 to 60 minutes. During these tests, the exhaust air from the suit was sampled for viable microorganisms at the outlet orifice of the pressure regulator, which was at the end of a 30-foot exhaust hose. After the test period, the microorganisms shed into the tank were collected and grown on appropriate culture media for subsequent enumeration. The data on microorganisms collected in the tank are shown in table 2.

Table 2

Microbial Contamination from Occupied, Pressurized Space Suit in a Static Shedding Chamber

Subject	Test Period (Minutes)	Total Microorganisms Shed	Mean Microorganisms Shed/Minute
I	30	16,100	537
	30	25,600	853
	45	2,900	97
	45	4,300	143
	60	2,200	73
II	30	2,300	77
	45	2,400	80
	45	4,400	146

Data on microorganisms collected from the outlet of the exhaust-air system were too erratic to be evaluated with confidence. Numbers of microorganisms collected in the tank (table 2) were not significantly different from the numbers collected in tests to estimate shedding from external contamination of the space suit (table 1). Thus, use of an open-loop air system did not, in these tests, permit an accurate estimation of total viable organisms shed through the suit; many microorganisms that normally could be retained and concentrated within the suit for potential penetration were carried outside the chamber with the exhaust air.

Average base-line rates of microbial dissemination for both subjects while dressed in sterile surgical scrub suits were within "normal" limits during controlled studies in the microbiotank; subject I shed about 13,000 organisms/minute and subject II about 4,800 organisms/minute.

In an attempt to obtain more precise quantitative data on microbial leakage from inside the space suit, a technique was developed for artificial introduction of tracer organisms into the suit. Adjusted quantities of a common yeast (Saccharomyces cerevisiae) were nebulized into the unoccupied, pressurized space suit. The suit was vertically supported inside the dynamic plastic chamber and pressurized to 18.4 psia, using a closed-loop system. The suit and chamber were purged for about 20 minutes before nebulization began. A hose line from the nebulizer was attached to one connector at the waist and a probe from a bacteriological sampler to another. Other bacteriological air samplers were used for sampling the air inside the plastic chamber. Measured quantities of yeast were nebulized into the pressurized suit at a constant rate for a 30-minute period.

Sampling results showed that the quantity of viable tracer organisms suspended inside the suit and the microbial leakage from the suit remained constant during the period of nebulization. The cell size range recovered inside the suit and the shedding chamber was 0.8 to 12+ microns, with a mode of 1.4μ. The results of 2 tests are shown in table 3.

Table 3

Contamination with Artificially Seeded Microorganisms from Unoccupied, Pressurized Space Suit in a Dynamic Shedding Chamber

Test	No. of Suspended Viable Organisms in Suit/Ft^3	No. of Viable Organisms Leaking through Suit/Minute
1	15,000	3,000
2	10,600	2,300

If the inside volume of the unoccupied, pressurized suit is assumed to be 4 ft^3, then the data in table 3 indicate that approximately 5% of the viable yeast cells inside the suit leaked out per minute.

Measurements of leakage of air from the suit tested were made before and after the studies summarized in this report. The results are shown in table 4.

Table 4

Rate of Leakage of Air from Space Suit

Test Location	Before Study	After Study
NASA Houston	1.1 Liters/Minute	2.5 Liters/Minute
CDC Savannah	————	2.5 Liters/Minute

As a result of the various preliminary studies reported above, techniques were developed to determine approximate rates of shedding of microorganisms from occupied and unoccupied space suits. Viable microorganisms shed from humans or artificially seeded into the suit tested leaked from this suit into the ambient environment. The rates of dissemination observed during these preliminary studies may have been related to the rates of microbial shedding or to the physical activity of the particular subjects used, or to variable rates of leakage of air from the suit, or a combination of these and other factors.

Reduction of Microbial Dissemination -- Studies on Microbial dissemination from humans were continued. Comparative data were obtained using the microbiotank during 30 tests with one male subject dressed in the following types of clothing:

(1) Sterile scrub suit (suit, cap, and socks), prepared and sterilized in the laboratory.

(2) As in (1), but wearing a sterile, disposable, pressed-fiber surgical face mask.

(3) Clean-room "bunny suit" (suit, hood, and sterile socks). Bunny suits were processed by a commercial laundry specializing in washing and delinting clean-room garb.

The subject donned clean undergarments following a nightly shower using a hexachlorophene detergent just before retiring. Daily scheduling of test attire was randomly determined for the 30 evaluations. All evaluations were performed during 30-minute test periods beginning at approximately 8 A. M. Pre-test R. H. in the microbiotank was reduced to approximately 25% at 68F. Under the static test conditions in the microbiotank, the R. H. increased steadily to about 85% maximum, at an average temperature of 70F. Results are shown in table 5.

Table 5

Shedding of Viable Particles by a Subject Dressed in Surgical and Clean-Room Clothing

Evaluation Number	Microbial Particles Shed Per Minute		
	Type of Garb		
	Sterile Scrub Suit with Cap and Socks	Sterile Scrub Suit with Cap and Socks plus Mask	Bunny Suit, Hood, and Sterile Socks
1	250	313	57
2	257	307	100
3	363	140	116
4	500	243	173
5	3,130	217	720
6	1,000	480	413
7	320	533	163
8	1,070	573	127
9	387	483	280
10	580	362	187
Averages	786	362	234

The data indicate that a significant reduction of microbial dissemination occurred while dressed in the bunny suit as compared with the scrub suit without masking.

Germicidal Activity of Ethylene Oxide -- Studies were continued on the activity of gaseous ethylene oxide against spores of *Bacillus globigii* laden on institutional dust particles. Test procedures using a static system for exposing these spores on glass surfaces to ethylene oxide were the same as reported earlier (Fourth Quarterly Report). The present studies were carried out at approximately 120F ± 0.5F. at an initial humidity of 50% ± 1.0%. A preconditioning period of 1 hour at 50% R. H. to moisten the spore material was used during some tests. Various gas concentrations were obtained at input pressures of 50 or 60 inches Hg absolute (1.7 to 2.0 atmospheres) using a mixture of 12% ethylene oxide and 88% Freon 12. Gas concentrations varied from 580 to 750 mg/liter during the test series, as measured by gas chromatography; concentrations remained essentially constant during each test.

Determination of final gas concentrations in the chamber on the basis of weight of gas dispensed was again demonstrated to be less accurate than use of gas chromatography. Failure to achieve sterility was again observed, although significant reductions of viable spores were obtained as shown in table 6. The delayed germination of surviving spores during some tests may indicate that exposure to the gas concentrations used caused only cell damage to these organisms. Spore material from 35 recent tests using concentrations of ethylene oxide of 750 to 1590 mg/liter are currently under assay for 50 days.

Reduction of Microbial Contamination on Surfaces -- Construction has been completed on a large, portable, dynamic chamber for use in laboratory or field studies on the reduction of microorganisms held under environmental conditions of 50C and 40% relative humidity. Following instrumentation, the system will be functionally evaluated and standardized for use. It is planned to isolate naturally contaminated areas within the environmental chamber for subsequent assay and evaluation of microbial death rates following varying exposure periods.

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Table 6

Effect of Gaseous Ethylene Oxide at 120 F on Dust Particles Laden with B. *globigii* Exposed for Varying Time Periods

Test Number	Exposure Time (Hours)	Weight of Gas Change (Pounds)	Pressure of Gas Change (in. Hg. Absolute)	Concentration of Ethylene Oxide Gas	Preconditioning Relative Humidity (Percent)	Exposure Relative Humidity (Percent)		Number of Slides Exposed	Mean Number of B. *globigii* Particles Exposed/Ft^2	Number of Slides Yielding Growth (48 Hrs.)	Mean Number of Viable Particles Remaining/Ft^2
						Start	Finish				
103	6	7.1	60	750	10	50	32	10	2.7×10^4	1*	14
104	18	5.4	50	580	10	50	20	10	4.9×10^4	1	4
105	6	7.1	60	750	10	50	31	10	5.9×10^4	1*	4
106	18	6.9	60	750	10	50	23	10	2.6×10^4	0	0
107	6	5.8	50	600	50	50	29	10	2.9×10^4	0	0
108	18	6.8	60	740	50	50	32	10	2.4×10^4	1*	4
109	6	6.9	60	750	50	50	37	10	1.2×10^5	0	0
110	6	6.8	60	750	50	50	48	10	3.8×10^4	0	0
111	18	6.6	60	750	50	50	26	10	6.8×10^4	0	0

*Growth after 50 days incubation at 37 C.